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Effect of smoking on the lipid composition of lung lining fluid and relationship between immunostimulatory lipids, inflammatory cells and foamy macrophages in extrinsic allergic alveolitis

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Effect of smoking on the lipid composition of lung lining fluid and relationship between immunostimulatory lipids, inflammatory cells and foamy macrophages in extrinsic allergic alveolitis. D.A. Hughes, P.L. Haslam.
ABSTRACT: Normal lung lining fluid suppresses lymphoproliferative responses. This effect is mediated by the major phospholipid components, but minor lipid components can stimulate lymphocyte proliferation. The aim of this study was to discover whether the changes in lung lipid composition reported in patients with extrinsic allergic alveolitis (EAA) might influence the levels of lymphocytes which occur in the lungs of these patients. Since cigarette smokers are less susceptible to EAA, we also investigated the effect of smoking on the lipid composition of lung lining fluid. Lung lining fluid was sampled by bronchoalveolar lavage (BAL) from 15 patients with EAA, and 9 nonsmokers and 13 smokers without lung disease. The smoking controls had increases in phosphatidylethanolamine, sphingomyelin and phosphatidylglycerol, but lower levels of cholesterol and cholesterol:total phospholipid ratios compared with the nonsmoking controls. By contrast, the patients with EAA had increases in total phospholipid and sphingomyelin; there were no smoking related decreases in cholesterol; and several patients had levels of cholesterol and cholesterol:total phospholipid ratios above the upper limit for the controls. In the BAL fluids of the EAA patients, the levels·ml^{-1} of the immunostimulatory lipids sphingomyelin, phosphatidylethanolamine, cholesterol and cholesterol esters correlated with the number·ml^{-1} of lymphocytes, mast cells, neutrophils and "foamy" macrophages. Cholesterol levels (r_s=0.82) and lymphocyte counts (r_s=0.90) correlated most closely with "foamy" macrophages ($p<0.001$), suggesting that uptake of cholesterol by macrophages may enhance antigen-presenting function. These observations provide some support for the hypothesis that inflammatory reactions in the lungs might be influenced by the local lipid environment.
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Extrinsic allergic alveolitis (EAA) (synonym - hypersensitivity pneumonitis) is an inflammatory granulomatous response of the lungs to a wide range of inhaled organic antigens [1]. Lung biopsies from the patients demonstrate increased numbers of lymphocytes infiltrating the alveolar walls and septa [2], and samples washed from the peripheral air spaces contain strikingly increased numbers of T-lymphocytes [3, 4] which appear to be activated [5]. The factors determining susceptibility to this disease are still poorly understood [6].

The air spaces of the lungs are lined with a lipid rich material containing components with surfactant properties which prevent alveolar collapse at low lung volumes [7]. JOUANEL *et al.* [8] reported that patients with EAA have alterations in the lipid composition of their bronchoalveolar lavage (BAL) fluid, most notably a striking decrease in the levels of the major phospholipid class found in the pulmonary surfactant system, phosphatidylcholine, with increases in phosphatidylethanolamine and cholesterol. We have recently reported that the lipid fraction of normal lung lining fluid obtained by BAL from humans, pigs and rabbits can suppress lymphocyte proliferation in a dose dependent manner [9] and ANSFIELD *et al.* [10] have reported similar findings using dogs. Furthermore, we have shown that while some lipid classes, including phosphatidylcholine and phosphatidylglycerol, suppress the induction of lymphocyte responses, others, namely cholesterol, sphingomyelin and phosphatidylethanolamine, augment them [11, 12]. We have also shown that altering the relative proportions of

mixtures of these lipids *in vitro* can modulate the levels of immunosuppression [11]. It is, therefore, possible that the alterations in the lipid composition of the lung lining fluid reported in patients with EAA may lead to a reduction in its immunosuppressive properties and favour the development of localized lymphoproliferative responses to inhaled antigens. The aim of this study was to test this hypothesis by examining whether the lipid composition of lung lining fluid obtained by BAL from patients with EAA has any relationship with the numbers of cells, particularly lymphocytes, recovered simultaneously in the fluid. We have also investigated the effects of smoking on lung lining fluid in view of the reports that EAA is a disorder which appears to be more prevalent in nonsmokers [13–15].

Methods

Subjects

Patients with EAA. Bronchoalveolar lavage samples were obtained from 15 patients with EAA, all of whom had histories of exposure to organic dusts and exposure-related respiratory symptoms. Nine of the patients had episodic symptoms (breathlessness with or without febrille episodes) whilst six had chronic breathlessness. Specific precipitating antibodies to antigens of the dusts were present in the serum of all patients (avian in 11 and moulds in four), and all but one had been exposed up to the time of admission for lavage. All had shown a reduction in lung function, in particular diffusing capacity for carbon monioxide (<80% mean predicted), although one patient had normal lung function at the time of lavage. All of the patients had shown diffuse nodular shadows on their chest radiographs, although two had normal radiographs at the time of lavage. One also had linear shadows suggestive of fibrosis. None were receiving treatment at the time of lavage. The mean age was 45±14 yrs (7 males, 8 females). Eight patients had never smoked and seven were cigarette smokers (3 ex-smokers and 4 current smokers).

Controls. As controls for this study, BAL samples were obtained from a group of nine healthy volunteers who had never smoked (seven males, two females, mean age 36±6 yrs). To evaluate the effect of cigarette smoking, a group of 13 current smoking controls were also studied (11 males, two females, mean age 44±18 yrs). These subjects had normal chest radiographs and were undergoing bronchoscopy for investigation of cough or minor haemoptysis. No abnormality was detected in any case. Ethical approval was obtained for all lavage studies, and all patients and controls gave their informed consent.

Bronchoalveolar lavage (BAL)

BAL samples were obtained from each individual studied by the method that we have previously described in detail [16] except that since 1987 we have employed a standardized 240 ml (4×60 ml) lavage fluid introduction volume, subject to clinical constraints. Several earlier samples were also included and the introduction volume ranged from 180–360 ml. The mean (±SD) introduction volume was 240±0 ml in the nonsmoker controls, 263±71 ml in the nonsmokers with EAA, 300±36 ml in the smoker controls and 291±54 ml in the smokers with EAA. The mean (±SD)% fluid recovery was 60±11% in the nonsmoker controls, and 43±6% in the nonsmokers with EAA, 46±19% in the smoker controls, and 46±16% in the smokers with EAA. Although the mean fluid recovery volume was higher in the non-smoker controls, there were no significant differences either in introduction volumes or % fluid recovered between the groups (Mann-Whitney U-test). The lavage procedure was performed using an Olympus fibreoptic bronchoscope introduced by the transnasal route after premedication with papaveretum and atroprine and under local anaesthesia with lignocaine. Supplementary oxygen was administered throughout the procedure. The tip of the bronchoscope was wedged in the lateral segment of the right lower lobe and lavage was performed using 4×60 ml aliquots of normal saline buffered to pH 7.0 with 8.4% sodium bicarbonate and prewarmed to 37°C. The aspirated fluid was collected into a sterile siliconized container and transported on ice immediately to the laboratory. The BAL fluid was centrifuged at 300 g for 10 min at 4°C to sediment the cells, and the supernatant fluid was divided into aliquots and immediately frozen and stored at -70°C. This separation procedure was completed within 20 min post-lavage to prevent the fluid from becoming contaminated with lipids from the membranes of cells which, if kept for more prolonged periods in saline, subsequently start to die *in vitro*. Our values for cell viability, assessed by trypan blue exclusion within 20 min, were >98% for all cell types except contaminating ciliated and squamous epithelial cells from the airways. These stained with trypan blue but rarely exceeded 5% of the total cells present.

Lipid analysis

Aliquots of BAL supernatant were thawed at room temperature and analysed by the methods that we have described previously [17]. Total lipid extraction was carried out by the methanol-chloroform method of BLIGH and DYER [18]. The organic phase containing the lipids was separated off and taken down to dryness under reduced pressure at 20°C in a rotary evaporator, resuspended in chloroform-methanol (9:1 v/v) to 40 mg lipid per ml, and stored under nitrogen at -70°C. The phospholipids present in each sample were determined using the improved one dimensional thin layer chromatography (TLC) system developed by GILFILLAN *et al.* [19], using silica gel plates (20 cm + 20 cm, K6; Whatman, Clifton, NJ) and chloroform-methanol-petroleum ether (bp 35–60°C) - acetic acid - boric acid (40:20:30:10:1.8 vol/vol/vol/vol/wt) as the developing solvent. The lavage extracts were run simultaneously alongside standard preparations of purified phospholipids

of known concentrations. After the plates were dried, the lipids were visualized by charring at 180°C [20], and the plates were read by a Shimadzu CS-920 TLC Scanner. Lipid phosphorus determinations of the spots were also carried out using the method of ROUSER *et al.* [21] to verify the results of the charring technique. The amount of each phospholipid present and its proportion of the total phospholipid content was then calculated for each sample by comparison with the reference standards. Total phospholipid yield was calculated by relating the sum of the amounts of the individual phospholipids present to the amount of total lipid applied to the TLC plate. The yields of cholesterol and cholesterol esters were determined using the same methods but using hexane diethylether - acetic acid (80:20:1.5 vol) as the developing solvent [22].

All solvents used were chromatographic standard (BDH, UK; Aldrich, UK) and all lipid standards were obtained from Sigma, UK. The efficiency and reproducibility of the method was confirmed by controls using known mixtures of pure lipid standards added to the saline solution used for the lavage procedure. This also confirmed that the improved one-dimensional method gave clear resolution of phosphatidylglycerol from phosphatidylethanolamine.

Lavage cell analysis

Total counts of the nucleated cells present in the lavage fluids were made by staining an aliquot of the cell suspension with 1% crystal violet in 1% acetic acid and counting the cells in an Improved Neubauer Counting Chamber. Results were expressed as the total number of cells·ml^{-1} of recovered lavage fluid. For differential cell counting, cytocentrifuge slide preparations of the cells were made in a Shandon Cytospin 2 (Shandon Southern Instruments, UK) using 100 µl aliquots of lavage cell suspension (2×10^6 cells·ml^{-1}), air-dried, then stained with May-Grünwald Giemsa stain, using methanol as the fixative and DPX as the mountant. Differential counts were made by counting at least 300 cells in random fields and expressing each cell type as a percentage of the total cells present. Aliquots of cells from each lavage sample were also fixed and prepared for examination by electron microscopy as we have reported previously [23].

Effect of esterified cholesterol compared with unesterified cholesterol on lymphoproliferative responses

We have previously reported the effects on lymphoproliferation of most of the major lipid components found in the lining fluid [11]. However, we have not previously studied the effects of cholesterol oleate, which has been observed within histiocytic foam cells in hypercholesterolaemic states [24]. Therefore, the effects of cholesterol and cholesterol oleate on the proliferative response of normal peripheral blood mononuclear cells (PBMN) to the mitogen phytohaemagglutinin (PHA) were tested by the methods that we have previously described in detail [11]. Briefly, PBMN were obtained from the defibrinated blood samples of six healthy adult volunteers by density centrifugation over lymphocyte separation medium (Flow Laboratories). The interfacial cell layer was washed twice and counted. The cells were resuspended at 0.5×10^6 cells·ml^{-1} in RPMI 1640 containing 25 mM Hepes buffer (Gibco) supplemented with penicillin (100 IU·ml^{-1}), streptomycin (100 µg·ml^{-1}, Gibco) and insulin, transferrin, selenium, linoleic acid, and bovine serum albumin (CR-ITS, Flow Labs). This serum-free medium was used because foetal calf serum is an abundant source of lipids, particularly cholesterol. One ml aliquots of the cell suspension were cultured in triplicate at an optimal concentration of PHA (0.5 µg·ml^{-1} PHA-P, Wellcome) in 6 ml polypropylene tubes (Falcon 2063). Dose response studies were performed using the following concentrations of cholesterol or its ester: 0.05, 0.1, 0.2, 0.4 and 0.8 mg·ml^{-1}. The lipids were dispersed into culture medium by sonication on ice. Tritiated thymidine (New England Nuclear) was added 18 h before harvesting. At 72 h the cell suspension was divided in 200 µl volumes into 96-well microtitre plates and harvested using an Ilacon harvester. Results were expressed as a percentage of the tritium counts obtained from cultures performed in the absence of the neutral lipids (mean±SEM). Viability was assessed by trypan blue exclusion.

Statistical methods

For the analysis of quantitative data, groups were compared using the Mann-Whitney U-test for non-parametric data distributions and correlations were made using the Spearman rank correlation coefficient. Analysis of the effect of lipids on lymphoproliferation was performed using the Wilcoxon matched-pairs signed-ranks test for pairs of related samples. P values (two-tailed) of ≤ 0.05 were accepted as significant and trends at $p \leq 0.1$ are also shown.

Results

Lavage lipid analysis

Nonsmoking and smoking control groups (tables 1 and 2). The smoking control group had significantly higher levels (µg·ml^{-1}) of total phospholipid·ml^{-1} in BAL fluid compared with the nonsmoking controls ($p<0.02$), due to increases in phosphatidylglycerol ($p<0.05$) and phosphatidylethanolamine ($p<0.002$) and sphingomyelin ($p<0.02$). By contrast, the smokers had significantly lower levels of the neutral lipid cholesterol ($p<0.05$). When the phospholipid class was expressed as a percentage of total phospholipid (table 2), phosphatidylethanolamine ($p<0.02$) and sphingomyelin ($p<0.02$) were also higher in the smokers. The cholesterol: total phospholipid ratio was significantly lower $p<0.0002$; (table 2). Six of the 13 smokers (46%)

Table 1. – Concentrations of lipids ($\mu g \cdot ml^{-1}$) in BAL fluid from nonsmoker and smoker control subjects

	Nonsmokers (9)		Smokers (13)	
	Median	Range	Median	Range
Phospholipids				
Total phospholipid	9.3	5.3–25.1	14.8**	10.4–37.8
Phosphatidylcholine	7.4	2.9–15.6	7.7	6.1–15.6
Phosphatidylglycerol	1.4	0.8–3.5	2.2*	1.0–6.6
Phosphatidylethanolamine	0.8	0–2.1	2.6***	0.6–6.4
Sphingomyelin	0	0–2.3	0.7**	0.1–5.3
Phosphatidylinositol	0	0–1.7	0	0
Phosphatidylserine	0	0–0.1	0	0–0.9
Lysophosphatidylcholine	0	0	0	0
Cardiolipin	0	0	0	0–3.9
Neutral lipids				
Cholesterol	6.4	1.9–9.6	2.6*	0.9–8.7
Cholesterol esters	4.4	1.3–5.7	3.2	0–9.5

*: $p<0.05$; **: $p<0.02$; ***: $p<0.002$ compared with nonsmoker control subjects (Mann-Whitney U-Test). BAL: bronchoalveolar lavage.

Table 2. – Relative proportions of different lipid classes in BAL fluid from nonsmoker and smoker control subjects

	Nonsmokers (9)		Smokers (13)	
	Median	Range	Median	Range
% of Total Phospholipid				
Phosphatidylcholine	65.4	52.1–86.6	53.6**	41.2–74.3
Phosphatidylglycerol	17.0	7.0–24.5	17.5	9.6–21.1
Phosphatidylethanolamine	8.2	0–18.2	19.2**	4.0–22.3
Sphingomyelin	0	0–13.3	4.7**	0–17.6
Phosphatidylinositol	0	0–9.3	0	0
Phosphatidylserine	0	0–0.9	0	0–7.4
Lysophosphatidylcholine	0	0	0	0
Cardiolipin	0	0	0	0–10.6
CH:PL	0.57	0.20–1.30	0.19***	0.03–0.54
CE:PL	0.42	0.20–0.50	0.23	0–0.70

**: $p<0.02$; ***: $p<0.002$ compared with nonsmoker controls (Mann-Whitney U-Test). CH: cholesterol; PL: total phospholipid; CE: cholesterol esters; BAL: bronchoalveolar lavage.

also had cholesterol ester:total phospholipid ratios below the lower limit of the range for nonsmokers (not shown in table). The proportion of the major phospholipid class, phosphatidylcholine, was significantly reduced in the smokers reflecting the increases in other classes (table 2), but the actual levels of phosphatidylcholine were not decreased (table 1).

Nonsmoking and smoking EAA patients (tables 3 and 4). The results for the nonsmoking and smoking patients with EAA have been separately compared with those for the nonsmoking and smoking controls to aid in distinguishing disease-related changes from those related to smoking. Unlike the findings for the controls, there were no significant differences either in concentrations (table 3) or relative proportions of lipids (table 4) between the nonsmokers and smokers with EAA.

However, the nonsmokers with EAA (table 3) had slightly higher concentrations of total phospholipid and a significant increase in sphingomyelin ($p<0.002$) compared with the nonsmoker controls. There was no significant difference in cholesterol levels, but one of the nonsmoking patients had an elevated cholesterol level

Table 3. – Concentrations of lipids ($\mu g \cdot ml^{-1}$) from nonsmokers and smokers with EAA compared with controls

	Nonsmokers				Smokers			
	Controls (9)		EAA (8)		Controls (13)		EAA (7)	
	Median	Range	Median	Range	Median	Range	Median	Range
Phospholipids								
Total phospholipid	9.3	5.3–25.1	15.6#	8.2–26.5	14.8	10.4–37.8	18.5*	12.1–28.4
Phosphatidylcholine	7.4	2.9–15.6	9.1	3.6–17.3	7.7	6.1–15.6	9.6#	8.1–15.1
Phosphatidylglycerol	1.4	0.8–3.5	2.4	1.1–4.6	2.2	1.0–6.6	4.1**	2.4–4.6
Phosphatidylethanolamine	0.8	0–2.1	1.5	0–2.4	2.6	0.6–6.4	2.7	0.8–4.5
Sphingomyelin	0	0–2.3	2.0***	1.1–3.3	0.7	0.1–5.3	2.7#	0.1–5.4
Phosphatidylinositol	0	0-1.7	0	0–0.3	0	0	0	0
Phosphatidylserine	0	0–0.1	0	0–1.3	0	0–0.9	0	0–2.7
Lysophosphatidylcholine	0	0	0	0–2.0	0	0	0	0
Cardiolipin	0	0	0	0	0	0–3.9	0	0
Neutral lipids								
Cholesterol	6.4	1.9–9.6	7.6	2.8–15.7	2.6	0.9–8.7	9.7**	3.3–19.5
Cholesterol esters	4.4	1.3–5.7	5.3	2.6–15.3	3.2	0–9.5	6.2	0.1–19.0

#: $p<0.1$; *: $p<0.05$; **: $p<0.02$; ***: $p<0.002$, compared with controls (Mann-Whitney U-Test). EAA: extrinsic allergic alveolitis.

Table 4. – Relative proportions of different lipid classes in BAL fluid from nonsmokers and smokers with EAA compared with controls

	Nonsmokers				Smokers			
	Controls (9)		EAA (8)		Controls (13)		EAA (7)	
	Median	Range	Median	Range	Median	Range	Median	Range
% of total phospholipid								
Phosphatidylcholine	65.4	52.1–86.6	56.4*	41.4–67.1	53.6	41.2–74.3	52.5	40.3–66.9
Phosphatidylglycerol	17.0	7.0–24.5	16.0	10.3–34.6	17.5	9.6–21.1	17.8	13.2–26.3
Phosphatidylethanolamine	8.2	0–18.2	9.2	0–22.8	19.2	4.0–22.3	14.5**	4.7–17.0
Sphingomyelin	0	0–13.3	13.9***	8.7–23.7	4.7	0–17.6	16.5#	0.6–23.4
Phosphatidylinositol	0	0–9.3	0	0–3.5	0	0	0	0
Phosphatidylserine	0	0–0.9	0	0–7.4	0	0–7.4	0	0–9.7
Lysophosphatidylcholine	0	0	0	0–11.4	0	0	0	0
Cardiolipin	0	0	0	0	0	0–10.6	0	0
CH:PL	0.57	0.20–1.30	0.52	0.21–0.77	0.19	0.03–0.54	0.42**	0.21–0.69
CE:PL	0.42	0.20–0.50	0.42	0.15–0.96	0.23	0–0.70	0.37	0.001–0.82

#: $p<0.1$; *: $p<0.05$; **: $p<0.02$; ***: $p<0.002$, compared with controls (Mann-Whitney U-Test). CH: cholesterol; PL: total phospholipid; CE: cholesterol esters; BAL: bronchoalveolar lavage; EAA: extrinsic allergic alveolitis.

and three had cholesterol ester levels above the upper limit of the range for the nonsmoker controls. The smokers with EAA (table 3) had a significant increase in total phospholipid ($p<0.05$) and a slight increase in sphingomyelin compared with the smoker controls. There was also a significant increase in phosphatidylglycerol ($p<0.02$) and a slight increase in phosphatidylcholine. Levels of cholesterol ($p<0.02$) were significantly higher and four patients had levels above the control range while two also had elevated cholesterol ester levels. In terms of relative proportions (table 4), sphingomyelin was higher ($p<0.002$) and phosphatidylcholine lower ($p<0.05$) in the nonsmokers with EAA compared with the nonsmoker controls. The smokers with EAA also had a slight increase in the proportion of sphingomyelin compared with the smoker controls (table 4). The proportions of phosphatidylethanolamine (table 4) were lower than in the smoker controls ($p<0.02$), but still higher than in either of the nonsmoking groups. The smokers

Table 5. – Cellular content of BAL fluid from nonsmokers with EAA compared with nonsmoker controls

	Controls (8)		EAA (8)	
	Median	Range	Median	Range
Total cells·ml^{-1}×10^4	9.8	3–22	42***	22–224
Macrophages·ml-1×10^4	8.3	2–21	15′	3–34
Differential count %				
Macrophages	84	70–98	21***	11–81
Lymphocytes	6	2–19	66***	13–82
Mast cells	0	0–0.1	0.5*	0–2.4
Neutrophils	3	0–10	6	2–47
Eosinophils	0	0–2	0.8	0–5.8

′: $p<0.1$; *: $p<0.05$; ***: $p<0.002$ compared with nonsmoker controls. BAL: bronchoalveolar lavage; EAA: extrinsic allergic alveolitis.

Table 6. – Cellular content of BAL fluid from smokers with EAA compared with smoker controls

	Controls (13)		EAA (7)	
	Median	Range	Median	Range
Total cells·ml^{-1}×10^4	47	9–127	128**	31–252
Macrophages·ml^{-1}×10^4	45	9–126	27	5–81
Differential count %				
Macrophages	95	81–99	19***	14–45
Lymphocytes	0.8	0–14	63***	33–70
Mast cells	0	0–0.5	3***	0.8–6.7
Neutrophils	0.8	0.2–3	13***	4–38
Eosinophils	0	0–2	0.6	0–3

**: $p<0.02$; ***: $p<0.002$ compared with smoker controls. BAL: bronchoalveolar lavage; EAA: extrinsic allergic alveolitis.

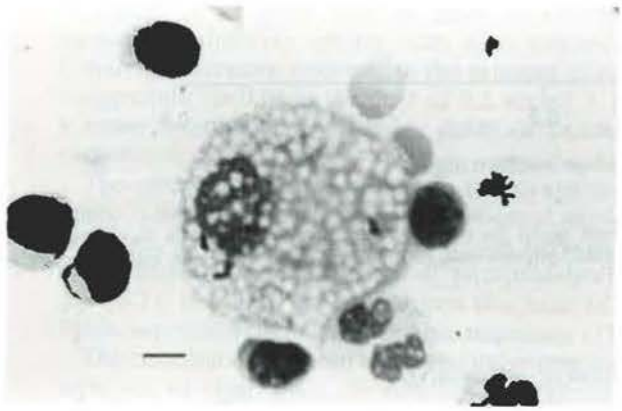

Fig. 1. – Alveolar macrophage with a foamy cytoplasmic appearance in a cytocentrifuge preparation of BAL cells from a patient with EAA. Methanol fixation/May-Grünwald Giemsa stain; Bar=5 μm. BAL: bronchoalveolar lavage; EAA: extrinsic allergic alveolitis.

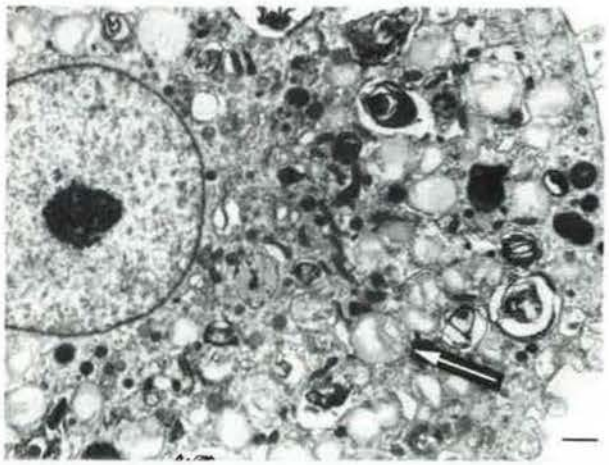

Fig. 2. – Ultrastructure of BAL "foamy" macrophage showing numerous neutral-lipid containing inclusions in the cytoplasm (arrow). Bar=1 μm. BAL: bronchoalveolar lavage.

Table 7. – Table showing correlation between BAL lipid levels (μg·ml^{-1}) and cell numbers (cells·ml^{-1}) in the 15 patients with EAA

	Cell numbers					
	Lymphocytes	Mast cells	Neutrophils	Eosinophils	Total macrophages	"Foamy" macrophages
Lipid levels	r_s	r_s	r_s	r_s	r_s	r_s
PC	0.22	0.33	0.08	-0.31	0.51*	0.43#
PG	0.15	0.22	0.19	-0.43#	0.17	0.23
PE	0.74***	0.55*	0.65**	0.04	0.45*	0.63*
SM	0.63**	0.53*	0.51*	-0.17	0.70**	0.69**
PI	-0.04	-0.13	-0.18	0.01	-0.18	-0.13
PS	-0.15	-0.23	0.11	-0.16	0.35#	-0.06
LPC	-0.42	0.01	-0.17	0.07	0.22	0.05
CL	0.22	0.22	0.22	0.23	0.22	0.22
Cholesterol	0.68**	0.53*	0.48*	0.01	0.89***	0.82***
Cholesterol esters	0.60**	0.52*	0.37#	0.14	0.62**	0.66**

r_s: Spearman rank correlation coefficient, #: p<0.1; *: p<0.05; **: p<0.01; ***: p<0.001. PC: phosphatidylcholine; PG: phosphatidylglycerol; PE: phosphatidylethanolamine; SM: sphingomyelin; PI: phosphatidylinositol; PS: phosphatidylserine; LPC: lysophosphatidylcholine; CL: cardiolipin; BAL: bronchoalveolar lavage; EAA: extrinsic allergic alveolitis.

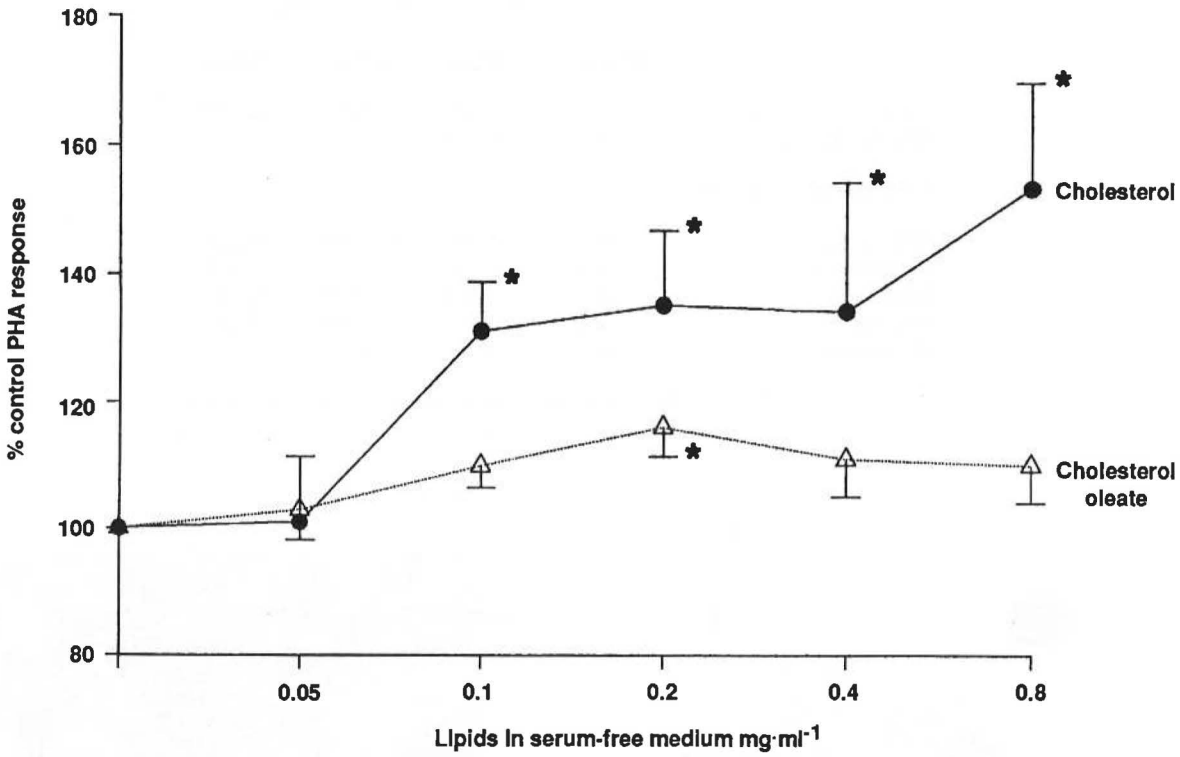

Fig. 3. – Effect of increasing concentrations of cholesterol and cholesterol oleate on the lymphoproliferative response to optimal PHA stimulation in serum-free media. Bars indicate standard error of the mean. PHA: phytohaemagglutinin. *: p<0.05 compared with control PHA response, Wilcoxon matched-pairs signed-ranks test.

with EAA had higher ratios of cholesterol:total phospholipid (p<0.02) compared with the smoker controls. Cholesterol ester:total phospholipid ratios were not significantly increased, but one smoker and four nonsmoker patients had values above the range for the respective control groups.

Lavage cell analysis

Lavage cell count data were available on all the subjects in this study, apart from one of the nonsmoking controls. Tables 5 and 6 show that, consistent with the diagnosis, the nonsmoking and smoking patients with

EAA had increased total numbers of cells·ml^{-1} and increased percentages of lymphocytes and mast cells compared with the nonsmoking and smoking control groups. The smokers also had increased percentages of neutrophils. The percentages of alveolar macrophages were decreased compared with the controls reflecting the increases in other inflammatory cells, although there were no significant decreases in the numbers of macrophages·ml^{-1}.

Many of the macrophages in the EAA patients (median 32%, range 6–59% of the macrophages in the nonsmokers and median 39%, range 28–76% in the smokers; no significant difference) had a "foamy" cytoplasmic appearance giving the impression of numerous small vacuoles of similar size in the cytoplasm (fig. 1). Irregularly sized vacuoles were seen in the cytoplasm of up to 12% of the macrophages in the control subjects, but less than 2% had the unusual "foamy" appearance. The foamy macrophages were examined by electron microscopy and numerous neutral-lipid containing inclusions were observed in the cytoplasm (fig. 2).

Correlations of lipids with inflammatory cells in the EAA group

Significant correlations were observed in the EAA patients between the concentrations·ml^{-1} of certain types of lavage lipids and the numbers·ml^{-1} of inflammatory cells present in the lavage samples (table 7). Levels of phosphatidylethanolamine, sphingomyelin, cholesterol and cholesterol esters correlated with the counts of all types of inflammatory cells, except eosinophils; and the findings were similar whether considering the total EAA patients or the nonsmokers and smokers separately. The closest correlations were with numbers·ml^{-1} of lymphocytes, total macrophages, and "foamy" macrophages. We have previously reported that phosphatidylethanolamine, sphingomyelin and unesterified cholesterol can stimulate lymphoproliferative responses [11], and in figure 3 we show that an ester of cholesterol, namely cholesterol oleate, can also enhance the lymphoproliferative response to the mitogen phytohaemagglutinin ($p<0.05$ at the dose of 0.2 mg·ml^{-1}), but to a lesser extent than comparable doses of unesterified cholesterol.

The other lipid classes present in the lavage samples (table 7) did not correlate with the numbers of any lavage cell type, except for a weak correlation between total macrophage counts and levels of phosphatidylcholine ($r_s = 0.51$; $p<0.05$). We have shown that most of these lipids suppress lymphoproliferative responses [11].

The correlations between lipids and inflammatory cells were not so clear when the results were expressed as relative proportions of lipids and the cell counts as differential percentages.

It was of interest that the numbers of lymphocytes (per ml) in the lavage samples from the EAA patients correlated more closely with the numbers of "foamy" macrophages per ml ($r_s=0.90$; $p<0.001$) than with the total counts of macrophages ($r_s=0.69$; $p<0.005$).

Discussion

We have used the technique of bronchoalveolar lavage to simultaneously examine both the lipid composition and cell contents of lung lining fluid from patients with EAA, in order to test the hypothesis that changes in the lipid composition of the fluid may affect its immunoregulatory properties *in vivo*.

The differences that we have observed in this study are unlikely to merely reflect differences in the volumes of fluid used for lavage because the introduction volumes and percentages of fluid recovered in the different groups did not differ significantly. Furthermore, differences in dilution will not invalidate the comparisons in this study between lipid levels and numbers of cells in the same lavage sample, since both are subject to the same dilution factor. The information on the relative proportions of different lipid classes in the lavage samples is another approach not influenced by variable BAL fluid dilution.

Our findings show that cigarette smoking has a marked influence on the lipid composition of lung lining fluid, inducing increases in the phospholipids phosphatidylethanolamine, sphingomyelin and phosphatidylglycerol, but causing a marked reduction in levels of the neutral lipid cholesterol and also in the ratio of cholesterol:total phospholipid. By contrast, the EAA patients had increases in sphingomyelin and cholesterol in the BAL fluids which could not be explained by differences in smoking habits.

JOUANEL *et al.* [8] reported that the proportion of the major surfactant phospholipid, phosphatidylcholine, is significantly decreased in EAA patients compared with controls. We observed slightly reduced proportions of phosphatidylcholine in our patients, however, the concentrations·ml^{-1} were not significantly reduced compared with our controls, which contrasts with the almost total loss of phosphatidylcholine observed by JOUANEL *et al.* [8]. This may be due to differences between our two studies in methods of phospholipid extraction, chromatographic separation and quantification. JOUANEL *et al.* [8] also failed to detect phosphatidylglycerol either in their control or patient lavage samples which may be due to the recognized difficulty of distinguishing phosphatidylethanolamine from phosphatidylglycerol using earlier chromatographic techniques [25]. Phosphatidylglycerol is now considered to be the second major phospholipid component of normal pulmonary surfactant [26, 27]. JOUANEL *et al.* [8] found striking increases in the proportions of phosphatidylethanolamine and phosphatidylinositol in their EAA patients compared with controls. We have not observed such disease-related increases in our EAA patients but have found marked increases in levels and proportions of phosphatidylethanolamine in cigarette smokers. JOUANEL *et al.* [8] gave no details of the smoking habits of the subjects in their study.

The most significant increase in our nonsmoking patients with EAA compared with the nonsmoking controls was in levels and proportions of sphingomyelin. A slight increase in sphingomyelin was also observed in the smokers with EAA, indicating an effect of disease superimposed upon the smoking-related increase in sphingomyelin. Sphingomyelin is found in cell membranes and is not generally regarded as being a true surfactant component [28]. It is found in much higher proportions in lung tissue than in pulmonary surfactant [19]. The elevation in this lipid both in patients with EAA and in smokers may, therefore, reflect an increase in cell turnover within the air spaces and/or local tissue damage. This may also explain the increases in phosphatidylethanolamine that we have observed in smokers, since phosphatidylethanolamine is also found in much higher concentrations in lung tissue than in lung lining fluid [19] or serum [29].

In a recent series of studies we have demonstrated that several of the lipid classes found in lung lining fluid, namely phosphatidylcholine, phosphatidylglycerol, phosphatidylserine and phosphatidylinositol, can suppress lymphocyte proliferation to the T-cell mitogen phytohaemagglutinin in a dose dependent manner, whereas others, namely phosphatidylethanolamine, sphingomyelin and cholesterol, can augment it [11]. We acknowledge that experiments using mitogens do not specifically examine antigen processing and presentation, however, accessory cells are required to induce lymphocyte responses to mitogen and they cannot be replaced by adding interleukin-1 to the culture [30, 31]. The reason why specific antigens were not used in these experiments is that serum-free culture medium had to be employed to avoid the effects from lipids present in serum, and the levels of lymphocyte response to specific antigens in serum-free culture medium were very low and variable. Nevertheless, our finding in this study of correlations between the numbers of lymphocytes, mast cells and neutrophils, and levels of immunostimulatory but not immunosuppressive lipids in lavage fluids from EAA patients provides some support for our hypothesis that lipids present in lung lining fluid may play a role in modulating inflammatory responses in the alveolar spaces. Esterified cholesterol can also be detected in lung lavage fluid, and in this study we have shown that cholesterol oleate also has an immunostimulatory effect, although the effect was less than that observed when comparable doses of unesterified cholesterol were tested. We are unaware of any descriptions of the fatty acid composition of cholesterol esters in normal human lung lining fluid. We chose to study the effect of cholesterol oleate on lymphoproliferation because it has been observed in high concentrations within histiocytic foam cells in hypercholesterolaemic rabbits [24], and in pulmonary foamy macrophages in a dystrophic strain of hamsters [32]. Also, SAHU and LYNN [33] characterized oleic acid as one of the major components (23% of the total fatty acids) of cholesterol esters isolated from BAL supernatants from patients with asthma. Whether other cholesterol esters have immunoregulatory properties remains to be investigated.

In agreement with JOUANEL *et al.* [8] we observed that several of our EAA patients had levels of unesterified cholesterol in the lavage fluids exceeding the upper limit for the controls. Furthermore, by contrast with the significant reductions in cholesterol we have demonstrated in smoking compared with nonsmoking controls, the smokers with EAA had significantly higher cholesterol levels and ratios of cholesterol:total phospholipid compared with the smoker control group. In relation to the apparent lower susceptibility of smokers to EAA [13–15], the smoking related reduction in lavage cholesterol levels and lower cholesterol:total phospholipid ratio we have demonstrated in our smoking control group is of interest. In studies of blood lymphocytes it has been shown that decreasing the ratio of cholesterol:phospholipid can increase cell membrane fluidity and cause a decrease in lymphoproliferation [34]. It is, therefore, possible that the lymphocytes in the alveoli of cigarette smokers are bathed in a fluid which may be relatively more immunosuppressive than that of nonsmokers. This could confer a lower susceptibility on the smokers to develop the localized cell-mediated hypersensitivity reactions which are thought to be involved in the pathogenesis of EAA. Indeed, it has been reported that BAL T-lymphocyte mitogen-induced proliferation is reduced in current smokers compared with nonsmokers [35, 36]. Increases in lavage lymphocytes are relatively infrequent in the disease idiopathic pulmonary fibrosis (synonym: cryptogenic fibrosing alveolitis) where demographic data has shown that 90% of the cases with desquamative interstitial pneumonia and 71% of those with usual interstitial pneumonia are cigarette smokers [37]. We have also found changes in lavage lipids in this disorder [17] but, unlike our findings in EAA, they were mainly reductions in phosphatidylglycerol. We have not observed any correlations between the levels of lipids and the levels of neutrophils and eosinophils (unpublished observations) which are the main inflammatory cell types in BAL in this chronic inflammatory lung disease, contrasting with the lymphocyte increases in EAA [23].

The correlation that we observed between levels of cholesterol and cholesterol esters and the numbers of foamy macrophages in the lavage samples of our EAA patients is also of interest. Foamy macrophages are a characteristic histopathological feature of EAA [2, 38] and cholesterol clefts are also frequently observed in the biopsies of EAA patients [2]. The correlations that we have demonstrated in lavage suggest that "foamy" macrophages may indicate uptake of cholesterol and cholesterol esters by alveolar macrophages in this disorder. This suggestion is also supported by the electron microscopy observations which demonstrated numerous neutral lipid inclusion bodies within the cytoplasm of the foamy macrophages in the BAL samples of our EAA patients. It has been reported that the induction of

xanthomas (infiltrates of lipid-containing histiocytic foam cells) in the dermis of hypercholesterolaemic rabbits correlates with serum cholesterol levels [24]. Also, incubation of human macrophages with immune complexes containing low density serum lipoproteins (LDL) increases LDL receptors on the macrophages which increases LDL uptake and promotes intracellular cholesterol ester accumulation and foam cell formation [39]. The reasons for accumulations of foam cells in EAA are not known, but while products of T-lymphocytes are implicated in the pathogenesis of the granulomatous reactions, Arthus reactions involving interactions of inhaled antigens with specific antibodies in the respiratory bronchioles and alveoli are thought to be involved in the acute stage influx of neutrophils [40]; and macrophages containing numerous globular vacuoles are a feature of experimentally induced Arthus reactions [41]. Foamy macrophages are also a recognized feature of the tissue response which occurs following bronchial obstruction [42], thus, it is also possible that they might arise in EAA as a consequence of bronchiolitis interfering with clearance mechanisms.

Alveolar macrophages in normal lungs have a relatively poor capacity to initiate lymphoproliferative responses compared with blood monocytes and macrophages from other sites in the body [43]. However, we have observed that alveolar macrophages from EAA patients have elevated levels of HLA-D region antigens on their surface, suggesting that they may have enhanced antigen-presenting function [44] as has been reported in the granulomatous lung disease sarcoidosis [45]. The observation in this study that numbers of lymphocytes show an especially close correlation with numbers of "foamy" macrophages in the BAL samples of EAA patients suggests that increased uptake or storage of immunostimulatory lipids such as cholesterol by alveolar macrophages may play a role in enhancement of local lymphoproliferative responses.

Although these observations are of interest in demonstrating correlations in EAA patients between the numbers of lymphocytes and other inflammatory cells and the levels of lipids with immunostimulatory properties in the BAL samples, it is not known whether the local increases in lipids play any role in the pathogenesis of EAA. It is known that subjects exposed to organic antigens who remain asymptomatic have increased levels of specific antibodies [46] and also increased numbers of lymphocytes in BAL compared to unexposed controls [47]. Whether asymptomatic exposed subjects have a similar lavage lipid composition to those in exposed patients must now be explored. The exact distinction between the local inflammatory pathways in asymptomatic compared with symptomatic exposed subjects remains a subject for continued investigation.

In conclusion, these findings in patients with EAA lend some support to the hypothesis that immunological responses within the lungs may be influenced by the immunoregulatory properties of the local lipid environment.

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References

1. Fink JN. – Hypersensitivity pneumonitis. *J Allergy Clin Immunol*, 1984, 74, 1–12.
2. Reyes CN, Wenzel FJ, Lawton BR, Emanuel DA. – The pulmonary pathology of farmer's lung disease. *Chest*, 1982, 81, 142–146.
3. Reynolds HY, Fulmer JD, Kazmierowski JA, Roberts WC, Frank MM, Crystal RG. – Analysis of cellular and protein content of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J Clin Invest*, 1977, 59, 165–175.
4. Leatherman JW, Michael AF, Schwartz BA, Hoidal R. – Lung T cells in hypersensitivity pneumonitis. *Ann Intern Med*, 1984, 100, 390–392.
5. Mornex JF, Cordier G, Pages J, Vergnon JM, Lefebvre R, Brune J, Revillard JP. – Activated lung lymphocytes in hypersensitivity pneumonitis. *J Allergy Clin Immunol*, 1984, 74, 719–728.
6. Keller RH, Swartz S, Schleuter DP, Bar-sela S, Fink JN. – Immunoregulation in hypersensitivity pneumonitis; phenotypic and functional studies of bronchoalveolar lavage lymphocytes. *Am Rev Respir Dis*, 1984, 130, 766–771.
7. King RJ. – Pulmonary surfactant. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1982, 53, 1–8.
8. Jouánel P, Motta C, Brun J, Molina C, Dastugue B. – Phospholipids and microviscosity study in bronchoalveolar lavage fluids from control subjects and from patients with extrinsic allergic alveolitis. *Clinica Chimica Acta*, 1981, 115, 211–221.
9. Wilsher ML, Hughes DA, Haslam PL. – Immunoregulatory properties of pulmonary surfactant: effect of lung lining fluid of proliferation on human blood lymphocytes. *Thorax*, 1988, 43, 354–359.
10. Ansfield MJ, Kaltreider HB, Benson BJ, Caldwell JL. – Immunosuppressive activity of canine pulmonary surface active material. *J Immunol*, 1979, 122, 1062–1066.
11. Wilsher ML, Hughes DA, Haslam PL. – Immunoregulatory properties of pulmonary surfactant: influence of variation in the phospholipid profile. *Clin Exp Immunol*, 1988, 73, 117–122.
12. Wilsher ML, Parker DJ, Haslam PL. – Immunosuppression by pulmonary surfactant: mechanisms of action. *Thorax*, 1990, 45, 3–8.
13. Warren CPW. – Extrinsic allergic alveolitis is a disease commoner in nonsmokers. *Thorax*, 1977, 32, 567–569.
14. McSharry C, Banham SW, Boyd G. – Effect of cigarette smoking on the antibody response to inhaled antigens and the prevalence of extrinsic allergic alveolitis among pigeon breeders. *Clin Allergy*, 1985, 15, 487–494.
15. Editorial. – Smoking, occupation and allergic lung disease. *Lancet*, 1985, i, 965.
16. Dhillon DP, Haslam PL, Townsend PJ, Primett Z, Collins JV, Turner-Warwick M. – Bronchoalveolar lavage in patients

with interstitial lung diseases: side-effects and factors affecting fluid recovery. *Eur J Respir Dis*, 1986, 68, 342–350.
17. Hughes DA, Haslam PL. – Changes in phosphatidylglycerol in bronchoalveolar lavage fluids from patients with cryptogenic fibrosing alveolitis. *Chest*, 1989, 95, 82–89.
18. Bligh EG, Dyer WJ. – A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*, 1959, 37, 911–917.
19. Gilfillan AM, Chu AJ, Smart DA, Rooney SA. – Single plate separation of lung phospholipids including desaturated phosphatidylcholine. *J Lipid Res*, 1983, 24, 1651–1656.
20. Bitman J, Wood DL. – An improved copper reagent for quantitative densitometric thin-layer chromatography of lipids. *J Liq Chromatog*, 1982, 5, 1155–1162.
21. Rouser G, Fleischer S, Yamamoto A. – Two dimensional thin-layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids*, 1970, 5, 494–496.
22. Cabot MC. – Hydrolysis of novel diacylglycerol analogs and phorbol diesters by serum lipase. *Biochim Biophys Acta*, 1985, 833, 330–335.
23. Haslam PL, Dewar A, Butchers P, Primett ZS, Newman-Taylor A, Turner-Warwick M. – Mast cells, atypical lymphocytes and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis: comparison with other interstitial lung diseases. *Am Rev Respir Dis*, 1987, 135, 35–47.
24. Ikeda M, Kodama H, Nohara N. – Process of foam cell formation in diet-induced hypercholesterolaemic rabbit and the Watanabe heritable hyperlipidemic rabbit. *J Dermatol*, 1987, 14, 305–312.
25. Poorthuis BJHM, Yazaki PJ, Hostetler KY. – An improved two dimensional thin-layer chromatography system for the separation of phosphatidylglycerol and its derivatives. *J Lipid Res*, 1976, 17, 433–437.
26. Pfleger RC, Henderson RF, Waide J. – Phosphatidylglycerol - a major component of pulmonary surfactant. *Chem Phys Lipid*, 1972, 9, 51–68.
27. King RJ. – Isolation and chemical composition of pulmonary surfactant. *In:* Pulmonary Surfactant. B. Robertson, L.M.G. Van Golde, J.J. Battenburg eds, Elsevier, Amsterdam, 1984, pp. 1–15.
28. Tria E, Barnabei O. – Lipoproteins and lipopeptides of cell membranes. *In:* Structural and functional aspects of lipoproteins in lining systems. E. Tria, A.M. Scanu eds, Academic Press, New York, 1969.
29. Thrall RS, Swendsen CL, Shannon TH, Kennedy CA, Fredrick DS, Grunze MF, Sulavik SB. – Correlation of changes in pulmonary surfactant phospholipids with compliance in bleomycin-induced pulmonary fibrosis in the rat. *Am Rev Respir Dis*, 1987, 136, 113–118.
30. Grey HM, Chestnut R. – Antigen processing and presentation to T-cells. *Immunology Today*, 1985, 6, 101–106.
31. Davis L, Lipsky PE. – Signals involved in T-cell activation. II. Distinct roles of intact accessory cells, phorbol esters and interleukin-1 in activation and cell cycle progression of resting T-lymphocytes. *J Immunol*, 1986, 136, 3588–3596.
32. Burbach JA, Langworthy TA, Schlenker EH, Frost-Stotz PM. – Lipidosis of pulmonary macrophages in the dystrophic hamster. *Exp Lung Res*, 1985, 9, 99–117.
33. Sahu S, Lynn WS. – Lipid composition of airways secretions from patients with asthma and patients with cystic fibrosis. *Am Rev Respir Dis*, 1977, 115, 233–239.
34. Ip SHC, Abrahm J, Cooper RA. – Enhancement of blastogenesis in cholesterol-enriched lymphocytes. *J Immunol*, 1980, 124, 87–93.
35. Daniele RP, Dauber JH, Altose MD, Rowlands DT Jr, Gorenberg DJ. – Lymphocyte studies in asymptomatic cigarette smokers. *Am Rev Respir Dis*, 1977, 116, 997–1005.
36. de Shazo RD, Banks DE, Diem JE, Nordberg JA, Baser Y, Bevier D, Salvaggio JE. – Bronchoalveolar lavage cell - lymphocyte interactions in normal nonsmokers and smokers. *Am Rev Respir Dis*, 1983, 127, 545–548.
37. Carrington CB, Gaenster EA, Coutu RE, FitzGerald MX, Gupta RG. – Natural history and treated course of usual and desquamative interstitial pneumonia. *N Engl J Med*, 1978, 298, 801–809.
38. Hensley GT, Garancis JC, Cherayil GD, Fink JN. – Lung biopsies of pigeon breeders' disease. *Arch Pathol*, 1969, 87, 572–579.
39. Griffith RL, Virella GT, Stevenson HC, Lopes-Virella MF. – Low density lipoprotein metabolism by human macrophages activated with low density lipoprotein immune complexes. *J Exp Med*, 1988, 168, 1041–1059.
40. Reynolds HY. – Hypersensitivity pneumonitis: correlation of cellular and immunologic changes with clinical phases of disease. *Lung*, 1988, 166, 189–208.
41. More RH, Movat HZ. – Cellular and intercellular changes in the Arthus phenomenon. *Arch Pathol*, 1959, 67, 679–693.
42. Spencer H. – Obstructive pneumonitis. *In:* Pathology of the Lung, Fourth Edn, H. Spencer ed., Pergamon Press, 1985, Vol 1, pp. 544–545.
43. Holt PG. – Down-regulation of immune responses in the lower respiratory tract: the role of alveolar macrophages. *Clin Exp Immunol*, 1986, 63, 261–270.
44. Haslam PL, Parker DJ, Townsend PJ. – Increases in HLA-DQ, DP, DR and transferrin receptors on alveolar macrophages in sarcoidosis and allergic alveolitis compared with fibrosing alveolitis. *Chest*, 1989, 1990, 97, 651–661.
45. Venet A, Hance AJ, Saltini C, Robinson BWS, Crystal RG. – Enhanced alveolar macrophage-mediated antigen-induced T-lymphocyte proliferation in sarcoidosis. *J Clin Invest*, 1985, 75, 293–301.
46. Patterson R, Wang JLF, Fink JN, Calvanico NK, Roberts M. – IgA and IgG antibody activities of serum and bronchoalveolar fluid from symptomatic and asymptomatic pigeon breeders. *Am Rev Respir Dis*, 1979, 120, 1113–1118.
47. Cormier Y, Bélanger J, Beaudoin J, Laviolette M, Beaudoin R, Hebért J. – Abnormal bronchoalveolar lavage in asymptomatic dairy farmers: study of lymphocytes. *Am Rev Respir Dis*, 1984, 130, 1046–1049.

Effet du tabagisme sur la composition lipidique du liquide de recouvrement alvéolaire. Relation entre les lipides d'immunostimulation, les cellules inflammatoires et les macrophages spumeux dans l'alvéolite extrinsèque allergique. D.A. Hughes, P. Haslam.

RÉSUMÉ: Le liquide de recouvrement alvéolaire du poumon normal supprime les réponses lymphoprolifératives. Cet effet est médié par les composants phospho-lipidiques majeurs, mais les composants lipidiques mineurs peuvent stimuler la prolifération lymphocytaire. L'objectif de cette étude est d'explorer si les modifications de composition des lipides pulmonaires observées chez les patients atteints d'alvéolite extrinsèque allergique (EAA) pourraient influencer les taux de lymphocytes observés dans le poumon de ces patients. Puisque les fumeurs de cigarettes sont moins sensibles à l'alvéolite extrinsèque allergique, nous avons exploré également l'effet de la fumée de tabac sur la composition lipidique du liquide de revêtement pulmonaire. Le liquide de

revêtement pulmonaire a été prélevé par lavage broncho-alvéolaire chez 15 patients atteints d'alvéolite extrinsèque allergique et chez 9 non-fumeurs et 13 fumeurs sans maladie pulmonaire. Chez les fumeurs, l'on a observé des augmentations de phosphatidylethanolamine, de sphingomyéline et de phosphatidylglycerol, et des diminutions du cholestérol et du rapport cholestérol/phospho-lipides totaux, par comparaison avec les contrôles non-fumeurs. Au contraire, les patients atteints d'alvéolite extrinsèque allergique avaient des augmentations des phospho-lipides totaux et de la sphingomyéline. L'on n'a pas observé de diminution de cholestérol en relation avec le tabagisme. Certains patients avaient des niveaux de cholestérol et de rapport cholestérol/phospho-lipides totaux au-dessus de la limite supérieure des sujets contrôle. Chez les patients atteints d'alvéolite extrinsèque allergique, les niveaux par ml des lipides immuno-stimulateurs (sphingomyéline, phosphatidylethanolamine, cholestérol et ester de cholestérol) étaient en corrélation avec le nombre par ml de lymphocytes, de mastocytes, de neutrophiles et de macrophages spumeux dans les liquides de lavage alvéolaire. Les niveaux de cholestérol (r_s=0.82) et les décomptes de lymphocytes (r_s=0.90) sont en corrélation la plus étroite avec les macrophages spumeux ($p<0.001$), suggérant que l'absorption de cholestérol par les macrophages pourrait stimuler la fonction de présentation des antigènes. Ces observations apportent quelque argument en faveur de l'hypothèse selon laquelle l'environnement lipidique local pourrait influencer les réactions inflammatoires pulmonaires.

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